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Transplantation of Bone-Forming Cells

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The transplantation of bone-forming cells includes the transfer of discrete pieces of viable bone and of *ex vivo* expanded osteoprogenitor cells. These progenitor cells, derived from bone marrow stroma, have been termed bone marrow stromal cells (BMSCs). BMSCs are pluripotent cells with several distinct phenotypes including bone, cartilage, fat, and muscle.

In vitro and in vivo analyses of BMSCs have been utilized to discern the influence of the donor and of growth factors on BMSC proliferation and differentiation. *In vitro* parameters utilized to evaluate cultured BMSCs include colony morphology, cell morphology, bone-specific and bone-related proteins, and enzyme expression. *In vivo* analyses historically have utilized diffusion chambers, implants beneath the renal capsule, and, most recently, subcutaneous implants.

In anticipation of their potential clinical applications, BMSCs have been used to close bone defects in animals and as vehicles for gene-mediated therapy. However, limited success to date in the latter effort suggests that further innovation and investigation is warranted. ■

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Introduction

Although the successful transplantation of osteoblasts and osteocytes has been accomplished by the transfer of discrete pieces of viable bone, the transplantation of *ex vivo* expanded osteoprogenitor cells is a relatively recent advance. These progenitor cells, derived from bone marrow stroma, have been termed bone marrow stromal cells (BMSCs). They are pluripotent cells with several distinct phenotypes. Recent research efforts have focused on characterization of these cells, development of techniques by which transplanted cells will form bone, and investigations into these cells' capacity to serve as vehicles for gene therapy.

Current Procedures for Bone Reconstruction

A discussion of bone-forming cell transplantation would be incomplete without at least a brief discussion of current procedures for the reconstruction and regeneration of bone; bone reconstruction currently is directed toward the repair of congenital bone defects and bone deficiencies after tumor extirpation or trauma.

Transplantation of Bone Graft

The clinical application of bone grafting is first credited to Macewen; in 1881 he reported the use of autogenous bone to reconstruct the humerus of a child [1]. Macewen suggested that the graft contained osteogenic cells that stimulated new bone formation. Later reports established the utility of this technique [2].

Despite the use of bone grafts for more than a century, investigators are continuing to characterize the means by which these grafts are responsible for new bone formation [3]. Implantation of a bone graft is first associated with the formation of a hematoma around the fragment of bone. A local inflammatory response is established, and the graft undergoes necrosis. The bone graft soon is resorbed by osteoclasts. Thus, although a small number of bone-forming cells from the graft may persist, they are likely not responsible for the majority of the graft's benefit. Instead, the graft's advantage to the recipient lies in its osteoconductive and osteoinductive properties [4].

When sufficient autograft bone has not been available, allogeneic bone has been used instead as a graft material. However, concern over the histocompatibility of fresh allograft led to the use of freeze-dried allografts [5]. Freeze-drying reduces both the immunogenicity and the viability of donor bone cells, such that these grafts provide only a limited osteogenic potential. The increased concern over

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the transmission of blood-borne diseases has further limited the use of allograft bone over the last decade.

Transfer of Osteogenic Cells Via Bone Marrow Transplantation

Other investigators have hypothesized that bone marrow contains osteogenic precursor cells [6, 7]. As a result, bone progenitor cells have been transplanted via transfer of whole bone marrow. Sharma percutaneously transplanted bone marrow into rabbit osteotomy defects and found improvement in callus volume and in healing after 4 weeks [8]. Clinical nonunions and delayed unions have been particularly amenable to percutaneous bone marrow injection. Healey and coworkers treated an oncogenic population that had undergone sarcoma resection followed by standard open bone grafting [9]. Of eight patients, five had received chemotherapy and one had received radiotherapy. All eight received an injection of autogenous bone marrow to the sites. Seven demonstrated bone formation, and five achieved bony union. Connolly described the healing of various tibial nonunions using percutaneously applied bone marrow; he noted that bone union occurred as quickly as would be expected with an open technique [10]. Complications were limited to a 2 day duration of discomfort in the donor site in most patients and a likely contusion of the posterior tibial nerve leading to transient dysesthesias in one patient. Most recently, Garg and coworkers demonstrated the healing of humerus, ulnar, and tibial fracture nonunions in 17 of 20 treated patients [11]. Five of the patients were not good candidates for an open procedure because they had poor skin quality; percutaneous grafting of bone marrow was perceived to be of great advantage.

Identification of BMSCs as Mediators of Skeletal Regeneration

Owen and Friedenstein demonstrated the presence of a population of bone marrow-derived stromal cells with an osteogenic capability [12]. These cells could be distinguished from the hematopoietic elements in the marrow by their high adherence to the substrate plastic in tissue culture flasks. Cultured BMSCs maintain many features consistent with pluripotency, including the ability to differentiate into several tissue types. *In vitro*, cultured human BMSCs synthesize collagenous and noncollagenous proteins that are components of normal skeletal matrix [13–15]. In the presence of dexamethasone and beta-glycerophosphate, cultured BMSCs will form mineralized nodules [16, 17]. After their expansion in tissue culture, these BMSCs from many different species are capable of forming new bone when transplanted subcutaneously in recipient mice [18, 19].

BMSCs are Precursors for Specific Tissue Types

Friedenstein first noted that cultures of BMSCs could form bone when transplanted under the renal capsule of mice [20]. Yet, his and other groups also observed that the tissues differentiating from BMSCs were not limited to bone. Hematopoiesis-supporting stroma, including adipocytes, cartilage, and sometimes even muscle have been described as resulting from the differentiation of BMSCs [21–23].

Ashton described the formation of both cartilage and bone when rabbit BMSCs were transplanted using a diffusion chamber model [23]. Bennett and colleagues cultured adipocytes from the bone marrow using rabbit serum and dexamethasone [22]. In transplanted diffusion chambers, these cells also developed bone. It was concluded that adipocytes in culture de-differentiated after transplantation; this lent support to the concept that bone marrow osteoblasts and adipocytes share a common precursor. Ferrari and colleagues demonstrated muscle regeneration by bone marrow-derived myogenic progenitors [21]. After transplanting cultured BMSCs in areas of muscle degeneration, they noted that these cells underwent myogenic regeneration. These results further support the concept that BMSCs are pluripotent cells that can differentiate along several different paths.

In Vitro *Characterization of BMSCs*

Numerous laboratories have attempted to characterize more specifically BMSCs, including those BMSCs demonstrating an osteogenic phenotype, and to determine the biochemical basis for the inherent heterogeneity that long has been noted in this population (Fig 1). However, although some parameters measured *in vitro*, as listed below, may be indicative of an osteogenic potential, the gold standard by which osteogenic capacity should be assessed is *in vivo* transplantation.

Colony Morphology

On initial plating of single cell bone marrow suspensions at low cell density, the stromal precursors from which BMSCs derive, the colony forming unit-fibroblasts (CFU-F), rapidly become adhesive to the tissue culture plastic. On addition of appropriate stimulatory factors, including irradiated feeder cells or special lots of fetal bovine serum, the individual CFU-Fs begin proliferating to form BMSC colonies. These colonies exhibit a broad range of growth habits; some colonies remain small, with only a few hundred

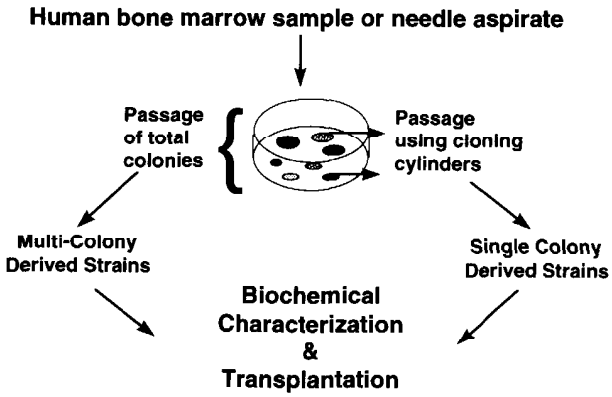


Figure 1. *Ex vivo* expansion of bone marrow stromal cells for biochemical characterization and *in vitro* transplantation. Single cell bone marrow suspensions obtained from either bone marrow aspirates or bone fragments are plated at low density in culture. Colony forming unit-fibroblasts begin to proliferate to form bone marrow stromal cell colonies. The resulting cultures can be passaged together to generate multi-colony-derived strains or individually to generate single colony-derived strains. The biochemical nature of each type of strain and its ability to form a bone/bone marrow organ on transplantation into immunocompromised mice then are characterized.

cells, whereas others form monolayer colonies of average (~1000 cells) and large (several thousands of cells) size. Furthermore, some average and large colonies form multiple layers of cells and, in some cases, nodular structures. These results indicate that CFU-Fs are stimulated to proliferate at different rates and that the nature of the proliferation (resulting in a monolayer vs. a multilayer vs. a multilayer with nodules) varies from one BMSC colony to another. The biochemical basis behind the differences in rate and nature of proliferation are not well known, but most likely it relates to the pattern of growth factor receptors that are expressed on individual BMSCs and the intracellular signal transduction pathways that they stimulate [24].

Furthermore, the phenotypic character of the colonies also varies. Some colonies display no particular phenotype, whereas others demonstrate 1) a pre-osteogenic or pre-adipogenic character, demonstrated by alkaline phosphatase activity; 2) a commitment to osteogenesis, indicated by calcium accumulation stainable with alizarin red; or 3) commitment to adipogenesis, demonstrated by fat accumulation and demonstrated by staining with Sudan Red (Fig. 2). It should be noted that not all types of colonies that are alkaline phosphatase positive and/or alizarin red positive are capable of regenerating bone *in vivo* (Satomura, Kuzetsov, Gehron Robey, unpublished results).

Cell Morphology

BMSCs within the individual colonies also exhibit a broad range of cell morphologies, ranging from flat and extremely large cells to a spindle-shaped fibroblastic morphology, with varying shapes and sizes in between. After passage of individual colonies, these differences in size and morphology sometimes are maintained. However, in multi-colony-derived strains, there is generally a gradual trend toward a more uniform, fibroblastic morphology by both single colony-derived and multi-colony-derived BMSC strains. This morphology is characterized by a large flattened cytoplasm and a large oval nucleus with prominent nucleoli. This switch to a more uniform morphology may be indicative of a reversion to a "default," or somewhat less committed, phenotype, resulting from the removal of BMSCs from the marrow microenvironment. In the marrow environment, the phenotypic state of the cells most likely is dictated by numerous signaling molecules such as growth factors, hormones, and other proteins.

Expression and Quantification of Bone-Specific and Bone-Related Proteins

Passaged, multi-colony-derived BMSCs have been found to express collagen Type I, collagen Type III, fibronectin, osteonectin, decorin, biglycan bone sialoprotein, and osteocalcin, with time in culture (Kuznetsov and Gehron Robey, unpublished results) [25–30]. They are not known to produce Factor VIII, indicating a distinction between these cells and endothelial cells, which are also pres-ent in the bone marrow environment [25, 26, 31–33].

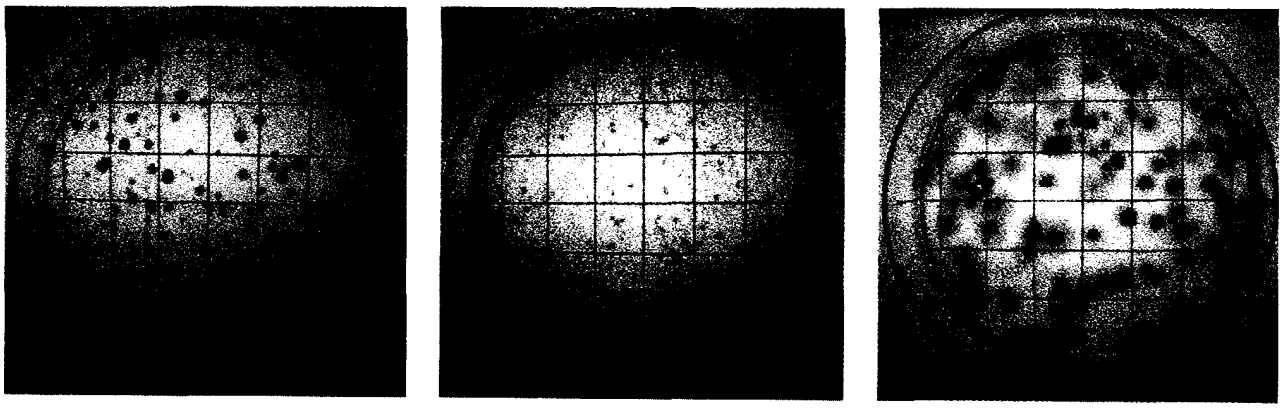
Enzyme Expression

BMSCs are known to express alkaline phosphatase; however, the level varies dramatically in individual colonies of BMSCs and from one animal species to another [26, 27, 29, 31–33]. They have little nonspecific esterase activity, distinguishing them from macrophages, and have almost no acid phosphatase activity [25–27, 29, 31–35]. They demonstrate minimal tartrate-resistant acid phosphatase activity, thereby distinguishing them from osteoclasts.

In Vivo *Demonstration of the Ability of BMSCs to Regenerate a Bone and Bone Marrow Organ*

Although *in vitro* analyses of bone-forming cells can provide clues as to their phenotypic potential, assessment of these same cells *in vivo* offers further insights into their behavior. Initial steps to characterize the behavior of cultured cells after transplantation in animal recipients utilized diffusion chambers [23, 36]. Here, cells were placed in a capsule and transplanted subcutaneously in mice or rats. The transplanted cells were separated from the host animal's cells by filters with a 0.45 micron pore diameter, a size that permitted the diffusion of macromolecules but prevented cellular or vascular invasion. When such sys-

HETEROGENEITY OF BMSCs
Phenotypic Expession



Alkaline Phosphatase (blue) (Other colonies red - eosin)

Fat Accumulation (Sudan Red)

Ca++ Accumulation (Alizarin Red)

Figure 2. Phenotypic heterogeneity of the bone marrow stromal cell (BMSC) colonies. Individual BSMC colonies exhibit different phenotypes, distinguished by various histochemical markers. All colonies stain with eosin, whereas varying percentages are stainable with Alkaline Phosphatase (A), indicative of pre-osteogenic and pre-adipogenic cells; Sudan Red (B), which identifies colonies that bear fat, indicative of more mature adipogenic cells; and Alizarin Red (C), indicative of Ca^{++} accumulation and a more mature osteoblastic phenotype. The percentage of the different phenotypes varies as a function of donor age and also as a function of certain disease states such as osteoporosis.

tems were used to transplant BMSCs, they formed bone and cartilage within 8 weeks. Although the behavior of the cells could be modulated by host proteins, growth factors, and other diffusable stimuli, the system was limited by its exclusion of any cellular infiltration by the host. In response to this shortcoming, Kuznetsov and Friedenstein transplanted BMSCs under the renal capsule in mice and noted bone formation within the transplants [37]. They characterized the bone as lamellar, with long trabeculae and abundant hematopoiesis. Although their system provided the first method for exposing BMSCs to *in vivo* cellular infiltration, its major shortcoming was its limitation to kidney transplantation. Such a model required considerable operative time and skill, and it limited the size of transplants. Krebsbach and coworkers extended Friedenstein's work by developing a system for transplant placement into the subcutaneous space of mice [18]. They identified a carrier that facilitated bone formation by BMSCs, and they developed a method for transplanting these constructs under the mouse skin. When mouse BMSCs were infiltrated into Gelfoam™ sponges before transplantation, they formed corticocancellous bone capsules that enclosed a well-defined hematopoietic compartment. Using *in situ* hybridization and immunohistochemistry, they confirmed that 8-week-old transplants derived their bone from donor cells while their hematopoietic elements came from the recipient animal. Transplantation of human BMSCs on Gelfoam™ carriers failed to produce significant bone; however, considerable human bone formed when human BMSCs were transplanted on hydroxyapatite/tricalcium phosphate particles. Bone, derived from the donor, and hematopoietic elements, derived from the recipient, formed in the spaces between the particles. Such a model may serve as the basis for the use of *ex vivo* expanded BMSCs to reconstruct bone deficits in patients.

Factors Influencing Bone Formation by *Ex Vivo* Expanded BMSCs

Donor Influence on the Harvest of BMSCs

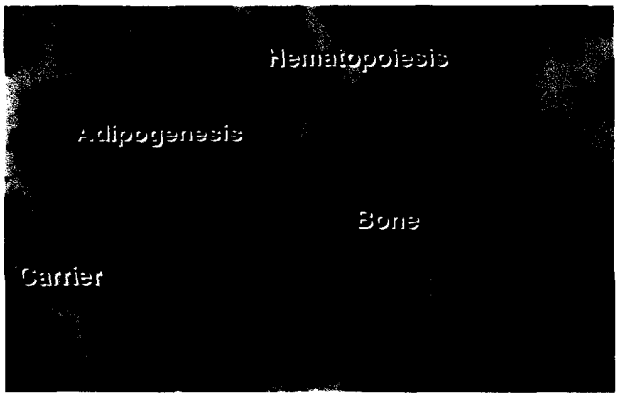
The yield of human BMSCs and assays of their *in vitro* mineralization potential have been shown to vary among donors. In general, it has been noted that the number of CFU-Fs declines with age in animal and human species. Additionally, individual BMSC colonies demonstrate a shift in their phenotypic expression pattern as a function of age. Muschler and coworkers assayed BMSC cultures from 30 patients of various ages for alkaline phosphatase activity [38]. They noted that the number of alkaline phosphatase-positive colonies (indicative of pre-osteogenic and pre-adipogenic colonies) declined with donor age but was unaffected by donor gender.

Ex Vivo *Expansion Culture Conditions*

It is clear from a variety of studies that the culture conditions under which BMSCs are expanded influence their ability to form a complete bone/bone marrow organ on *in vivo* transplantation. Biologic agents that influence the rate or quantity of formed bone include dexamethasone, fibroblast growth factor (FGF-2 and basic FGF), and transforming growth factor-beta 1 (TGF-β1).

Dexamethasone influences both the proliferative capacity of cells *in vitro* and their ability to form bone *in vivo* [39]. BMSCs were cultured in either the presence or absence of dexamethasone (10 nmol/L) and then transplanted intraperitoneally in mice in association with hydroxyapatite/tricalcium phosphate cubes (Fig. 3). Transplants from cells cultured with dexamethasone formed bone with active osteoblasts, whereas cells cultured in dexamethasone-free conditions produced only loose fibroblastic tissue. Interestingly, dexamethasone does not appear to influence bone formation if other factors are optimized, including the lot of fetal bovine serum in which the cells are cultured and the matrix in which the cells are transplanted [18].

Martin and coworkers have assessed the influence of FGF-2 that was added to human BMSC cultures at the time of initial plating [40]. Human BMSCs were cultured in the presence of only fetal calf serum or fetal calf serum plus each of the following growth modulators: EGF, PDGFaa, PDGFbb, FGF-2, GH, IGF 1, TGF β1, or dexamethasone. Cells from each experimental group then were seeded onto 64 mm [3] blocks of hydroxyapatite and transplanted into the subcutaneous space of immunodeficient mice. After harvest at 8 weeks, those cells cultured in FGF-2 or dexamethasone demonstrated 12.6% and 4.4%



(Cartilage only very rarely observed)

Figure 3. Bone formation by bone marrow stromal cells (BMSCs) transplanted along with a carrier into immunocompromised mice. Ex vivo expanded multi-colony-derived strains of human BMSCs were attached to a carrier consisting of hydroxyapatite/tricalcium particles and transplanted subcutaneously into immunocompromised mice. After 6 weeks, a complete bone/bone marrow organ is formed with 1) abundant new bone deposited on the ceramic particles; 2) active hematopoiesis of recipient origin; 3) supportive stroma of donor origin; and 4) associated adipogenesis of donor origin.

bone, respectively, whereas other growth factors were associated with insignificant quantities of bone.

Hanada and co-investigators tested the influence of bFGF and BMP-2 on the bone-forming ability of rat BMSCs [41]. Cells that had been exposed to both of these agents were loaded onto porous calcium phosphate cubes and transplanted *in vivo*. They formed more bone than those cells that had been pre-exposed to either agent alone.

Modulation of growth factors during *ex vivo* culture expansion of BMSCs can influence the degree of bone formation by these cells. Optimization of bone formation will be particularly important when BMSCs are transplanted in patients to reconstruct bone defects.

Effect of Carrier on Bone Formation

BMSCs must be transplanted in association with a carrier, or matrix, to form bone *in vivo*. To date, certain carriers have been identified as appropriate for bone formation by human BMSCs, whereas others have been found to be appropriate for mouse BMSCs but not for human cells.

When they first described their model for the subcutaneous transplantation of BMSCs, Krebsbach and coworkers specifically compared the bone-forming ability of mouse and human BMSCs using a variety of carriers, including collagen (Gelfoam™), polyvinyl sponges, porous collagen matrices, human demineralized bone matrix,

HA/TCP ceramics blocks, HA/TCP ceramic powder, and type I bovine fibrillar collagen strips [18]. Mouse BMSCs transplanted in collagen and polyvinyl sponge matrices formed a capsule of cortical-like bone that surrounded a core of active hematopoiesis. Consistent bone formation by human BMSCs was achieved only within carriers containing HA/TCP ceramics in the form of blocks, powder, and powder mixed with type I bovine fibrillar collagen strips (Collagraft™). Notably, bone formation was insignificant among transplants that included Gelfoam™ or polyvinyl sponges infiltrated with human BMSCs.

In accordance with these observations, other groups have achieved bone formation successfully by combining human BMSCs with HA/TCP blocks. Bruder and coworkers successfully loaded such blocks with human BMSCs and transplanted them into long bone defects in athymic mice, noting bone formation as early as 8 weeks [42]. Interestingly, Dennis and collaborators attempted to quantify the number of cells actually entering these blocks using ^{3}H-thymidine labeling. They found that exposing the blocks to 5 million cells resulted in only 45,000–65,000 cells actually penetrating and attaching to the block [43].

Animal Models That Have Been Used to Reproduce Human Clinical Conditions

The establishment of bone in heterotopic subcutaneous sites in animals provides a convenient means of assessing the osteogenicity of transplanted cells [18]. However, before this technology for bone formation can be offered to patients, its efficacy must be established in animals. To date, BMSCs have been transplanted in a variety of skeletal defects in mice, rats, and dogs.

Krebsbach and Mankani have transplanted cultured mouse BMSCs in critical-sized defects of the mouse calvarium [44] (Fig. 4). The aim of their investigation was to determine whether cultured allograft mouse BMSCs could close cranial defects in immunocompromised mice without the addition of exogenous growth factors or morphogenetic proteins. Transplanted cells came from a transgenic mouse carrying a procollagen type I-CAT construct, to distinguish host from recipient cells. A 5 mm cranial defect was prepared with a trephine burr in each recipient animal and filled with either Gelfoam™ sponges with BMSCs, Gelfoam™ sponges without BMSCs, Gelfoam™ sponges with splenic fibroblasts, or no transplants at all. Animals were examined for bone formation within the defects from 2 to 12 weeks after transplantation. Results from the histologic analysis are presented in Table 1. Marrow stromal cells were capable of osteogenesis leading to complete regeneration of the critical-sized cranial defect as early as 2 weeks after transplantation. In contrast, no significant bridging of the cranial defect with new bone was observed in animals receiving either no transplant, Gelfoam alone, or Gelfoam seeded with splenic fibroblasts. In these surgical sites, new bone formed occasionally but was limited to the margins of the defect.

Bruder and associates used cultured human BMSCs to close segmental defects in athymic rats [42]. Cells were loaded onto a tubed ceramic carrier that was placed into the defect. The bone ends were stabilized using internal fixation, and the animals were examined from 4 to 12

Figure 4. Bone regeneration by bone marrow stromal cells (BMSCs) in a critical-sized defect. Cultured bone marrow stromal cells were used to close a critical-sized calvarial defect in mice. Cultured mouse BMSCs were seeded onto Gelfoam™ sponges and placed in 5 mm diameter, critical-sized calvarial defects in immunocompromised mice. Within 4 weeks, new bone had formed and closed the defect. Hematopoiesis developed within the new bone capsule and was of recipient mouse origin.

Table 1
Osteogenesis by BMSCs in critical sized cranial defects (mean percentage of coverage of each calvarial defect)

Transplantation Procedure	2 weeks	3 weeks	4 weeks	6 weeks	12 weeks
BMSCs + Gelfoam™	99.0 ± 2.0	94.1 ± 6.3	99.1 ± 1.7	92.5 ± 18.4	100
Control 1[a]	nd	nd	3.7 ± 6.4	3.3 ± 5.2	6.7 ± 5.8
Control 2[b]	3.9 ± 6.7	0.0	0.0	4.0 ± 4.0	nd
Spleen fibroblasts + Gelfoam™	17.7	nd	14.8 ± 25.6	nd	nd

[a]Sham operated control; no Gelfoam™ sponge or cells added.
[b]Gelfoam™ sponge without BMSCs.
BMSC = bone marrow stromal cell; nd = not determined.

weeks after the operation. They reported that the cell-loaded ceramics contributed to increased bone formation and increased bone strength when compared with cell-free ceramics. Extending their work to larger animals, they demonstrated similarly encouraging results when repairing long bone defects in dogs [45].

In addition to the repair of discrete bony defects, BMSCs have the potential to aid in the formation of vascularized bone flaps. Such flaps offer the potential for operative bone transfer to avascular or otherwise inhospitable recipient sites because the bone segments are fully vascularized at the time of placement. Mankani and coworkers reported that transplantation of murine BMSCs around vascular pedicles into the neck of mice led to the formation of vascularized bone islands that derived their blood supply solely from the pedicle [46]. Casabona and coworkers described formation of an osteomyocutaneous flap from a myocutaneous flap by transplantation of BMSCs in a ceramic form into the latissimus dorsi muscle of mice [47].

Uses of Bone-Forming Cells in Gene Therapy

Osteoprogenitor cells also have considerable potential as: 1) vehicles for delivery of genes or growth factors to the bone; and 2) vehicles for the systemic expression of growth factors.

Because of their pluripotent nature, BMSCs have emerged as candidates for cell-mediated gene or growth factor delivery. Loaded into a carrier, these cells can be transplanted subcutaneously as discrete grafts, placed and removed with minimal invasiveness and left *in situ* only as long as they are needed. A small group of recent studies points to the possible applications of these cell transplants [48, 49].

BMSCs also may be useful for delivery of genes to fracture sites or areas of nonunion. Balian and coworkers transfected a clonal mouse cell from bone marrow stroma with a cDNA coding for IGF-1 and Neomycin [49]. These cells were introduced via intravenous and intramedullary routes into the femurs of recipient mice that had undergone an osteotomy. Serum IGF-1 increased gradually during the 4 week observation period, whereas the amount of Neomycin DNA first increased and then decreased. The transplanted cells were localized to the callus of the osteotomy. Although the increase in serum IGF-1 level is encouraging, the decrease in Neomycin DNA levels may indicate a decrease in the number of surviving cells. In the event this technique was utilized to speed or improve healing of fractures, transience of the growth factor augmentation may or may not be a disadvantage.

Allay and coworkers demonstrated the potential for gene expression by subcutaneously transplanted human bone marrow-derived stromal cells [48]. They isolated human BMSCs, transfected them with human IL-3 and LacZ, seeded the cells onto ceramic cubes, and transplanted these constructs into immunocompromised mice. They noted that the cells secreted IL-3 into the mouse circulation for as long as 12 weeks after transplantation.

Although direct transplantation of BMSCs shows considerable promise for local bone regeneration, their full potential as a therapy for generalized skeletal deficiencies or as a vehicle for gene transfer and treatment will not be realized without the development of techniques to introduce them systemically into multiple bone sites. Thus far, published reports specifically reflect the injection of either molecularly engineered or immortalized cells, their systemic dispersal, and their survival in multiple tissue sites, including bone and muscle [21, 49, 50]. However, the injection of normal, nontransformed BMSCs and their engraftment has been less successful to date [51, 52]. Laver and colleagues, for instance, analyzed the karyotype of bone marrow in six patients who had a received a bone marrow transplant from a sibling of the opposite gender at some time during the prior year. In these patients, all of whom had suffered from aplastic anemia or leukemia, all hematopoietic cells at the time of analysis were of donor origin, whereas all stromal cells were of recipient origin.

This suggested that although hematopoietic elements engrafted, such a phenomenon did not occur among the stromal cells. The absence of long-term engraftment by BMSCs and the lack of specificity of engraftment sites are phenomena that are not well understood. It is possible that cells may have to be modulated to: 1) allow for their survival in the circulation; and 2) direct their engraftment to specific organs.

Despite the difficulties outlined above, a clinical phase I trial assessing the safety of *ex vivo* expansion and subsequent autotransplantation of human BMSCs has been completed. Lazarus and coworkers aspirated bone marrow from 23 patients with hematological malignancies, expanded the cells *ex vivo* for intervals ranging from 4 to 7 weeks, and autotransplanted 1, 10, or 50 million cells in 15 of the 23 patients [53]. The patients were noted to have suffered no adverse reactions from the autotransplants.

Conclusions

Bone-forming cells include osteoblastic cells and differentiated progenitor cells arising from the culture of bone marrow stroma. Although transplantation of osteoblasts and osteocytes included in bone fragments has been performed for more than a century, the transplantation of osteoprogenitor cells is a relatively recent advance that requires the resolution of a number of practical issues. Studies have attempted to characterize these cells, delineate growth factors that promote the osteoblastic lineage, and identify matrices or carriers that facilitate bone formation *in vivo*. In parallel with these efforts, other studies have focused on techniques for using such cells as vehicles for gene-mediated therapies. Because many of these challenges are resolved through basic and translational investigation, clinical application of these potential therapies hopefully will provide patient benefit in the near future.

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Erratum

The following figures appeared in the November/December issue of *The Endocrinologist* in the article "Transplantation of Bone-Forming Cells" by Mahesh H Mankani, M.D., and Pamela Gehron Robey, Ph.D. Unfortunately, the appearance of these figures was not up to our usual standards. We regret the error. This is how the figures should have appeared.

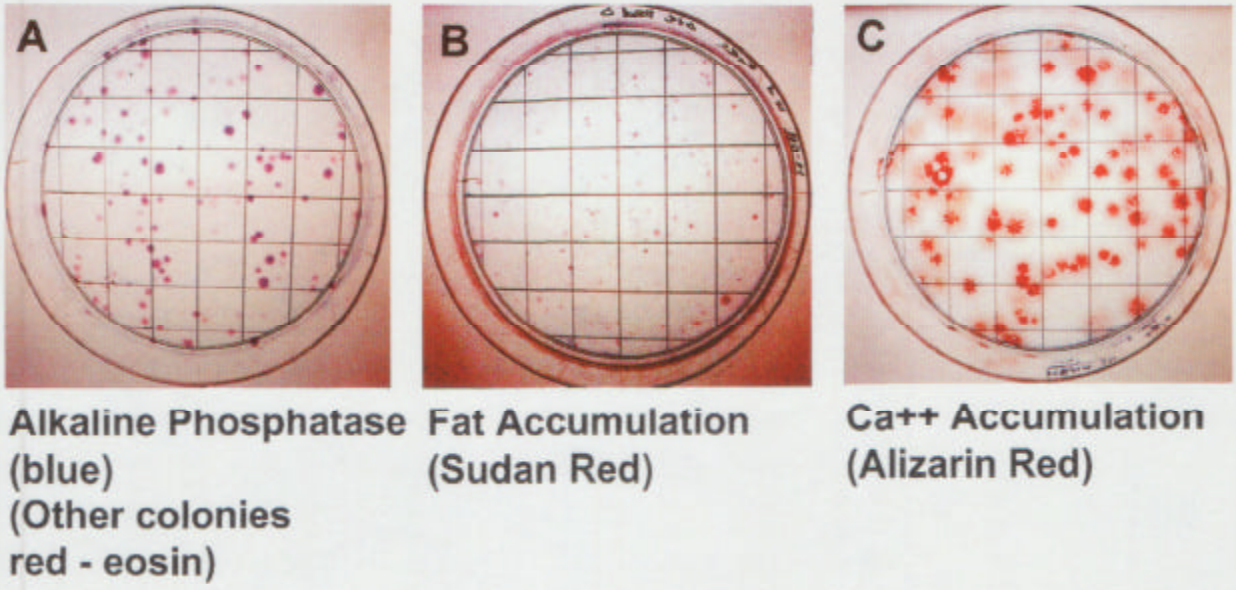

Figure 2. Heterogeneity of BMSCs: phenotypic expression. Phenotypic heterogeneity of the bone marrow stromal cell (BMSC) colonies. Individual BMSC colonies exhibit different phenotypes, distinguished by various histochemical markers. All colonies stain with eosin, whereas varying percentages are stainable with Alkaline Phosphatase (A), indicative of pre-osteogenic and pre-adipogenic cells; Sudan Red (B), which identifies colonies that bear fat, indicative of more mature adipogenic cells; and Alizarin Red (C), indicative of Ca^{++} accumulation and a more mature osteoblastic phenotype. The percentage of the different phenotypes varies as a function of donor age and also as a function of certain disease states such as osteoporosis.

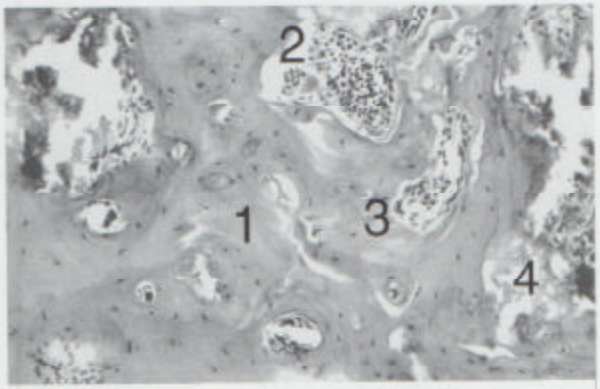

Figure 3. Bone formation by bone marrow stromal cells (BMSCs) transplanted along with a carrier into immunocompromised mice. *Ex vivo* expanded multi-colony-derived strains of human BMSCs were attached to a carrier consisting of hydroxyapatite/tricalcium particles and transplanted subcutaneously into immunocompromised mice. After 6 weeks, a complete bone/bone marrow organ is formed with a) abundant new bone deposited on the ceramic particles; b) active hematopoiesis of recipient origin; c) supportive stroma of donor origin; and d) associated adipogenesis of donor origin. 1 indicates bone, 2, hematopoiesis; 3, adipogenesis; and 4, carriet.

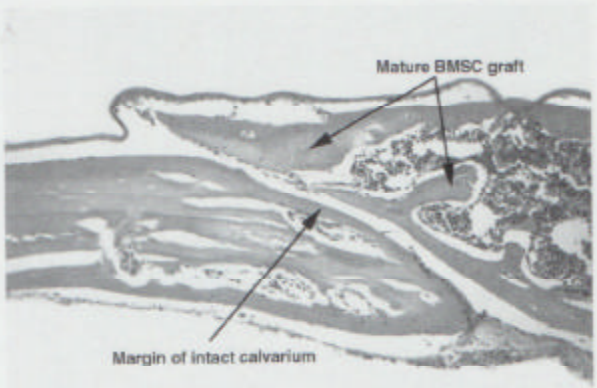

Figure 4. Bone regeneration by bone marrow stromal cells (BMSCs) in a critical-sized defect. Cultured bone marrow stromal cells were used to close a critical-sized calvarial defect in mice. Cultured mouse BMSCs were seeded onto Gelfoam™ sponges and placed in 5 mm diameter, critical-sized calvarial defects in immunocompromised mice. Within 4 weeks, new bone had formed and closed the defect. Hematopoiesis developed within the new bone capsule and was of recipient mouse origin.